3D imaging of biological specimens with and without optics

Tobias Beetz, Chris Jacobsen*, Janos Kirz, Onur Mentes, David Sayre, David Shapiro
Dept. Physics & Astronomy, SUNY Stony Brook

Chi-Chang Kao, Cecilia Sanchez-Hanke
National Synchrotron Light Source, Brookhaven National Laboratory

John Miao
Stanford Synchrotron Radiation Laboratory

*: presenting author (Chris.Jacobsen@sunysb.edu)

Several approaches can be used for 3D x-ray imaging of biological specimens. One approach is to use incoherent brightfield imaging to take a series of 2D images over a range of specimen angles for tomographic reconstruction; this approach has been used to obtain ~100 nm 3D images of frozen hydrated biological specimens (1, 2). However, because the depth of field $\sim\lambda/\theta 2$ decreases as the square of improvements in transverse resolution $\sim\lambda/(2\theta)$ as the numerical aperture θ is increased, this approach faces challenges if one attempts to use zone plate optics with higher resolution. For example, if 20 nm resolution optics are used at 500 eV, the depth of focus becomes only about 1 μm which is a thickness accessible to higher resolution experiments in energy filtered intermediate voltage electron microscopes. Futhermore, this approach is largely insensitive to the specimen's phase.

One approach to higher resolution 3D imaging experiments is to dispense with optics and simply record diffraction patterns of a non-crystalline specimen. This approach has been demonstrated in two dimensions (3), and efforts to extend this to three dimensional imaging of biological specimens are discussed in this workshop. We have developed a numerical model of a cell that allows one to explore the recording, assembly, and reconstruction of data from these sorts of experiments, and computer reconstruction experiments on the transition between weak phase and strong phase objects, and the use of low resolution zone plate images to aid in reconstruction, are presented by Shapiro *et al*. For thicker, more complex objects, we also propose to use diffraction tomography (4, 5) for three-dimensional imaging. By using zone plate optics to transfer a magnified version of in-line holograms to a soft x-ray CCD detector; we can obtain already-phased information from a complex object without any limitations due to depth of field of the zone plate optics. This approach of diffraction tomography has been demonstrated in visible light imaging (6), and hard x-ray imaging experiments have been carried out as well (7).

All of these approaches (tomography, diffraction, and diffraction tomography) require one to look at biological specimens at cryogenic temperatures so as to minimize the effects of radiation damage. We describe an experimental apparatus under design which is intended to allow ±80° rotation of a frozen hydrated specimen, and micron-resolution alignment of pinholes, zone plates, etc. both upstream and downstream of the object. Present plans call for the incorporation of an in-vacuum soft x-ray CCD camera for direct image detection. This apparatus will also be used for diffraction imaging of magnetic specimens.

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